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16. ABSTRACT

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SOME DETERMINANTS OF INTESTINAL CADMIUM TRANSPORT IN THE RAT[1,2]

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The hypothesis was tested that Cd absorption from the intestinal lumen is mediated by cellular transport systems. Cd is readily extracted from glucose-saline during perfusion of jejunal segments in the living rat. Over periods as long as 40 minutes, essentially all extracted Cd is recovered in the wall of the intestine. Cd uptake by the tissue obeys saturation kinetics with K_M values of the order of 0.1 mM, and V_{max} approximately 0.01 μmol/g/min. Although washing after exposure to ^{109}Cd removes only little radioactivity from the tissue, it reverses at least partly the saturating effects of higher Cd concentrations. Unidirectional flux of Cd into the tissue is inhibited by 10 mM Ca; no effect on backflux of Cd is seen. In contrast, Zn and EDTA both accelerate washout of Cd. The Ca content of skimmed milk fully accounts for the depressing effect of dried milk on Cd uptake. These results point to the presence in mucosal cell membranes of a saturable process responsible for Cd uptake and sensitive to inhibition by certain solutes in the lumen.

INTRODUCTION

Outside the occupational environment, ingestion represents the major route of human exposure to Cd. Net fractional absorption of the metal from the gut, however, amounts to only a few percent of the oral load (Moore *et al.*, 1973); the factors restricting absorption to such low values are not well understood. One important variable undoubtedly is diet and the presence in the intestinal lumen of dietary constituents and other compounds which might either directly affect systems involved in Cd transport, or indirectly exert their effect by reacting with Cd, thus altering its diffusibility and transport characteristics. Thus, chronic milk feeding was reported to increase Cd absorption in young rats (Kello and Kostial, 1977). A direct influence of intraluminal

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reactions of Cd is seen, for instance, in the work of Kojima and Kiyozumi (1974) and of Cherian *et al.* (1978). Effects of Cd chelators on epithelial Cd uptake have also been noted in the renal tubule (Foulkes, 1974).

Although it has been reported that movement of Cd out of the intestinal lumen obeys first order kinetics (Kojima and Kiyozumi, 1974), results of the present work suggest a different conclusion. Saturation of a hypothetical transport system, then, or its low affinity for Cd, could constitute additional determinants of Cd uptake. A suggestion that proteins similar to metallothionein may be involved in Cd absorption (Evans *et al.*, 1970) is attractive but unproven. Sugawara and Sugawara (1977) proposed a role for metallothionein in the well-documented prolonged retention of Cd in the intestinal wall; sloughing of mucosal cells with their metal content presumably also contributes to the low net Cd absorption into the body (Richards and Cousins, 1974).

Present understanding of reactions involved in intestinal Cd transport does not permit a full evaluation of all these possibilities. The work reported here was undertaken in order to explore further the contribution of various factors to Cd uptake from the lumen of the rat jejunum.

MATERIALS AND METHODS

Male Sprague-Dawley rats, weighing 250-300 g, were maintained on commercial chow (Purina) and tap water *ad lib* for at least one week before study. Twenty-four hours before the experiment, food was removed. Anesthesia was induced with Inactin (100mg/kg IP); body temperature was maintained close to 37°C by means of a rectal probe and a thermostatically-controlled heat pad. The trachea was cannulated. In some studies mean arterial blood pressure was determined in the femoral artery with a Harvard Instrument Co. transducer; in general, blood pressures remained normal throughout the studies. A 15 cm length of jejunum, starting at the ligament of Treitz, was cannulated at both ends and perfused at varying rates from a reservoir kept at 37°C. The problem of mixing and therefore of long periods (>20 minutes) required before effluent concentrations reached steady values interfered with use of physiologically reasonable flow rates (<0.1 ml/min). With a somewhat higher flow (0.4 ml/min, average perfusion pressure 2 cm H_2O) 5 minutes sufficed to attain a steady state which could thereupon be maintained for at least 30 minutes. As many as 4 different solutions could thus be tested sequentially over a period short enough to assure the stability of the preparation. In some studies, perfusion solutions were recirculated through the intestine. Inclusion of the reservoir in the dead-space volume necessitated further increases in perfusion rate. This procedure permits convenient sequential sampling for the determination of transport kinetics. Transit time of perfusate through the intestine was estimated with a small bolus of Dextran Blue in saline, and luminal volume was calculated as the product of perfusion rate and transit time.

Under no conditions did it prove practicable to perfuse more than 2 or 3 segments of intestine simultaneously, making difficult simultaneous comparison of effects of several variables. In one series of experiments, therefore, the

cannulated intestine was filled as usual; 3 cm sections were then tied off and the abdomen closed for 30 minutes (stationary incubation). Mixing of luminal contents in such short isolated segments proved inadequate for sequential sampling. After stationary incubation, therefore, the intestine was removed for analysis of contents and tissue in each segment, as well as for evaluation of Cd uptake by the remaining carcass in a Packard whole body counter.

The standard intestinal solution contained 5 mM glucose in saline, together with 0.1 μCi/ml ^{3}H-polyethylene glycol (New England Nuclear) as volume marker. Glucose was added because of its tendency, observed early in this study, to support constant Cd transport. $CdCl_2$ labelled with ^{109}Cd (0.02 μCi/ml) was added to the desired concentration, and removal of Cd from the lumen was calculated from changes in the ^{109}Cd/^{3}H ratio as determined on a Packard liquid scintillation spectrometer with automatic external standard. Rates are expressed as μmol Cd removed/unit weight or length tissue/minute. Tissues to be analyzed were first cut open, then rinsed for 10 seconds in saline, blotted on the serosal side, and finally weighed and measured. Cadmium content of the tissue was determined on a Packard well-type scintillation spectrometer. Knowledge of the relative counting efficiencies made it possible directly to relate disappearance of Cd from the lumen with its accumulation in the tissue. Glucose absorption from recirculating perfusate was measured with a glucose oxidase procedure.

RESULTS

Activity gradient along jejunum

Ideally one would wish to compare simultaneously effects of several variables on Cd removal from the lumen. To determine the feasibility of such a procedure, changes in the luminal ^{109}Cd/^{3}H ratio were measured in a series of contiguous 3 cm segments after a period of 30 minutes (stationary incubation). Fig. 1 shows results of 1 of 3 such studies.

An activity gradient along the intestine is clearly apparent, with the ability to remove Cd from the lumen mirrored by its accumulation in the tissue. Existence of this gradient makes it impossible to compare simultaneous activities in more than 2 contiguous segments. Study of 2 segments proved useful, provided each comprised about 1/2 of the average length employed for experiments similar to that shown in Fig. 1 and provided the order of addition of control or experimental solution was alternated. On this basis consistent results could be obtained in the study of a single variable, each animal serving as its own control (cf. Table 1).

Extent of Cd transport

The ability of the intestinal wall to accumulate and retain Cd is well documented. Under present conditions also, as seen in Fig. 1, Cd is accumulated in the tissue. In two studies the absolute loss of Cd from the lumen (perfused with 0.02 mM Cd) was calculated from the combined volume of

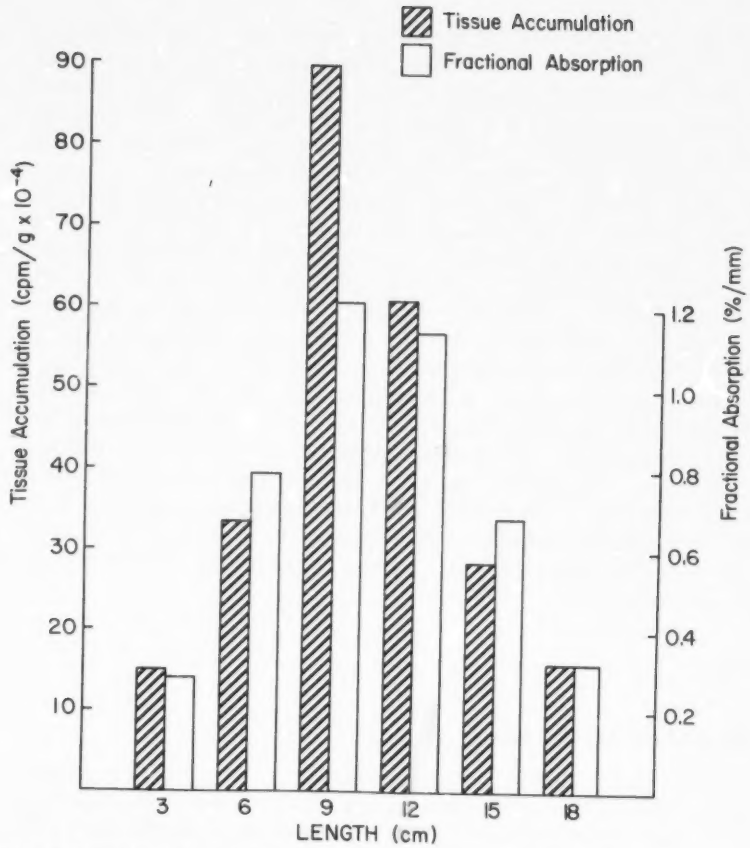

FIGURE 1. Stationary incubation (30 min) in contiguous jejunal segments. For details, see text.

TABLE 1. Effect of Milk on Cd Removal

Solution	n	Fractional Removal[a] (%)	Activity (% of control ± SE)
Saline	15	21 (range 10-48)	100
10 mM $CaCl_2$ in saline	5	12	55 ± 6
4% (w/v) SMP[b] in saline	10	10	50 ± 10

[a]Removal of Cd was measured during 30 minutes' stationary incubation in 2 contiguous segments, each animal serving as its own control; removal was normalized for tissue weight. [b]SMP: skimmed milk powder, containing 0.25 nmol Ca/g, i.e. final Ca concentration 10 mM. Concentration of ^{109}Cd: 0.02 mM.

luminal fluid recovered and saline washings, together with the $^{109}Cd/^{3}H$ ratio. Tissue recovery of Cd lost from the lumen amounted to 85 percent (experiment 1, 12 minute perfusion) and 79 percent (experiment 2, 40 minutes). In two further studies on 2 animals each, 1 ml of the usual 0.02 mM Cd solution was introduced into the jejunum. One animal in each pair was killed immediately and the gut removed; stationary incubation in the second animal continued for 30 minutes. As counted with the whole body counter, the ratio of carcass activity in animal 2/animal 1 never exceeded 1.0. In other words, no significant transmural movement of Cd occurred under present conditions.

Effects of milk

The stationary incubation technique described above was used to determine effects of some soluble dietary constituents (milk) on Cd removal from the lumen. Each segment contained 1 ml saline (glucose was omitted in these experiments); dried skimmed milk powder (40 mg/ml) was added randomly to one of the 2 contiguous segments under study. Table 1 illustrates the strong inhibition exerted by milk constituents. In each case, activity in the control segment simultaneously determined was equated to 100 percent. Fractionation of the milk powder showed no activity in the protein fraction isolated on Sephadex G75. Instead, full inhibitory power was retained upon wet ashing of the powder. Finally, as also shown in Table 1, the Ca content of the ash adequately accounted for the ability of milk acutely to interfere with Cd removal.

Ca inhibition of Cd flux

More detailed understanding of Cd translocation and its inhibition by Ca requires information on kinetics of the process. Fig. 2 illustrates the rapid removal of Cd from a recirculating perfusate; also shown are the Ca inhibition previously observed during stationary incubation and the saturating effect of high Cd concentration.

Ca inhibition of net Cd removal out of the lumen involves depression of flux from lumen to tissue, not accelerated washout of Cd from the tissue. This is illustrated in Fig. 3 by results of one of 3 similar studies in which intestines had been preloaded by perfusion for 20 minutes with 0.02 mM ^{109}Cd in saline-glucose as usual. The perfusate was then replaced with a Cd-free solution, and the ratio of residual ^{109}Cd to ^{3}H was equated to 100 percent. During the next 15 minutes only little ^{109}Cd was washed out of the tissue, as deduced from the slow rise in the isotope ratio. This control washout rate was not altered by addition of 10 mM Ca. The inactivity of Ca may be contrasted with the effects of Zn and EDTA, as determined in separate studies; both substances strongly accelerated washout. Zn not only accelerates washout of Cd, but also depresses the forward flux of Cd, as shown in experiments (not further detailed here) in which initial Cd removal from the lumen was measured in intestines not previously exposed to Cd.

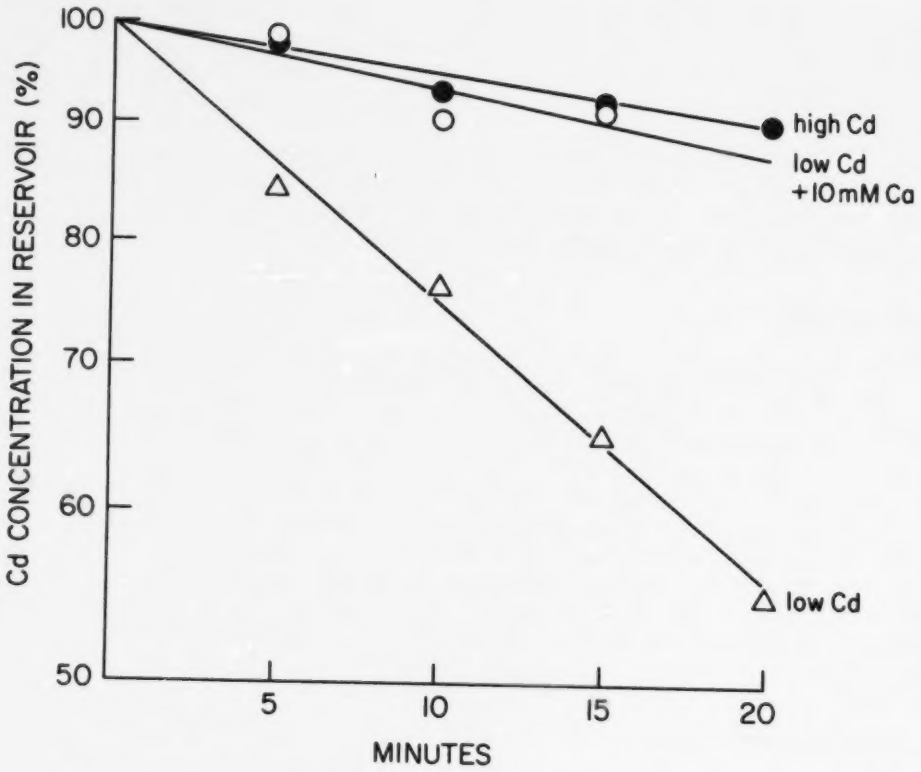

FIGURE 2. Time course of Cd uptake from lumen of perfused jejunum. Initial concentrations of Cd were 0.02 mM and 0.20 mM; total volume perfusate 3 ml, perfusion rate 0.6 ml/min, length tissue 22 cm.

Kinetics of Cd removal

Although reduced fractional Cd removal at higher Cd concentrations could be readily demonstrated in recirculation experiments (Fig. 2), somewhat more consistent results were obtained with steady state perfusion at 0.4 ml/min. Even with this technique, however, great individual variation was found, as shown in Fig. 4 by the results of three consecutive studies. Similar results were repeatedly obtained (Table 2). Although these further experiments all confirmed the saturability of Cd transport, only two Cd concentrations were studied in each, and they have, therefore, not been included in Fig. 4. What is very apparent in both Fig. 4 and Table 1 is the great variability between animals; this renders difficult attempts to define accurately the kinetic constants of Cd transport.

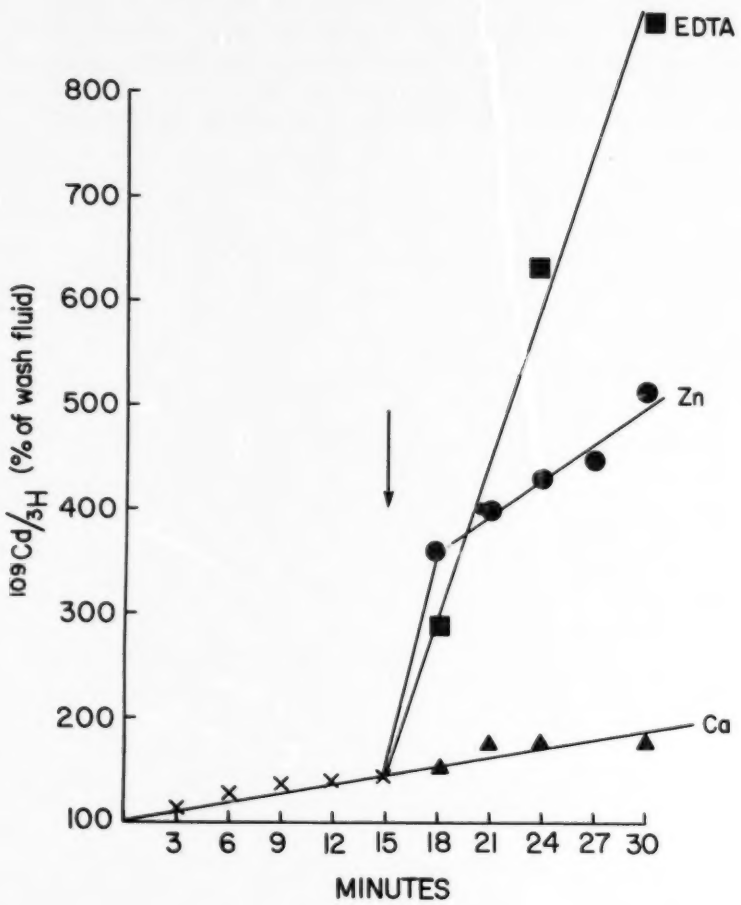

FIGURE 3. Reversal of Cd uptake from lumen. Tissue was preloaded with ^{109}Cd as described in text; perfusate was then diluted 20-fold with Cd-free solution, and the ratio of residual ^{109}Cd to ^{3}H equated to 100. After 15 minutes 10 mM Ca, Zn or 5 mM EDTA were added as shown.

To test the hypothesis that the effect of high Cd concentrations represents a non-specific intoxication of the system, two experimental approaches were used. In two studies removal of glucose from the perfusate was followed over a period of 25 minutes and found not to be reduced by 0.5 mM Cd. In another two animals, jejunum (28 cm) was filled with glucose-saline as usual. The outflow cannula was closed and the lumen connected to a horizontal pipette

whose emptying provided a direct measure of volume absorption. Control values of fluid absorption were 0.17 and 0.27 ml/minute compared to 0.21 and 0.25 ml/min after addition of 0.5 mM Cd. In other words, no acute effects of 0.5 mM Cd on intestinal function could be observed. Substitution of 5 percent mannitol for the saline completely abolished volume absorption and provided a positive control.

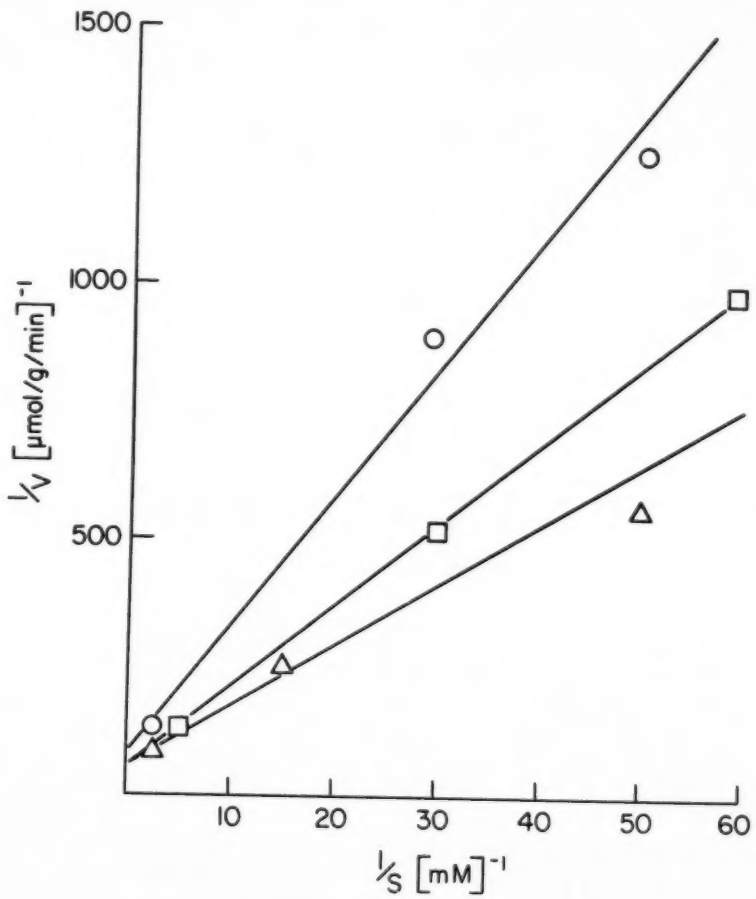

FIGURE 4. Kinetics of Cd absorption from lumen. Steady-state perfusion at 0.4 ml/min; results of 3 different animals are shown.

TABLE 2. Reversible Saturation of Cd Transport

Experiment #	Cadmium Transport					
	Period I (.02 mM)		Period II (nM)		Period III (.02 mM)	
	A	B	A	B	A	B
1	3.5	100	0	0	1.2	34
2	1.8	100	9.6	53	1.3	72
3	2.2	100	4.0	18	2.0	91
4	5.5	100	17.6	32	1.9	35
5	3.4	100	4.0	12	1.1	32
5	2.9	100	0	0	1.1	38
Mean		100		19		50

Perfusion rate 0.4 ml/min. Cd transport is expressed in Column A in nmol/g/min, in B as fractional absorption in percent of control. The initial Cd concentration for each period is also shown.

Reversibility of saturation effects

In nine experiments, jejunum was first perfused as usual at 0.4 ml/min with glucose-saline containing 0.02 mM ^{109}Cd, and the rate of Cd removal was determined. The perfusion solution was then replaced with glucose-saline containing 0.40 mM unlabelled Cd, a concentration adequate to severely depress fractional Cd transport. The tissue was allowed to accumulate Cd for 20 minutes before the perfusate was replaced with the original 0.02 mM Cd solution. Cadmium transport was now measured again and found to equal 51 percent (SD 29 percent) of the original control. In 4 further studies, the 0.4 mM Cd was omitted in period 2 in order to evaluate the influence of experimental procedures on the stability of Cd transport; in this case the mean activity in period 2 equalled 52 percent of that in period 1. Although these results were highly variable, they nevertheless suggest that the specific action of Cd in depressing, at higher concentrations, the fractional Cd absorption from the intestine, is largely reversible. Further evidence for reversibility was sought in 6 consecutive studies in which intestines were perfused at constant tracer concentration, first in presence of 0.02 mM Cd, then at 0.20 mM Cd, and finally again with the low Cd concentration. Results of these studies are collected in Table 2 and show that simple dilution can at least partially reverse the inhibitory effects of higher Cd concentrations.

DISCUSSION AND CONCLUSIONS

It is important to emphasize that results described in this paper do not refer to the transmural movement of Cd, but only to its transport from lumen into intestinal wall. The term *transport* is used advisedly, as simple physical diffusion cannot readily explain the saturation kinetics observed here. The rate of this transport is relatively fast, and it obviously does not limit transmural Cd uptake. Such a conclusion in turn implies that transfer of Cd into the body is determined by the rate of its release from the intestinal wall. Prolonged reten-

tion in the mucosal cells, as pointed out by others (e.g. Richards and Cousins, 1974) could lead to return of Cd to the lumen upon sloughing of cells. This fact presumably contributes to the generally low net absorption of Cd.

Characteristics of the process of Cd transport out of the lumen remain poorly defined. Under present conditions the process appears to obey saturation kinetics, with an approximate K_M of 0.1 - 0.2 mM (Fig. 4), and a V_{max} of the order of 0.01 μmol/g intestine/minute. Such a saturable process would contribute a major portion of total Cd absorption only at relatively low Cd concentrations, such as might be encountered under natural conditions. In contrast to present results, Kojima and Kiyozumi (1974) did not observe saturation kinetics and concluded that Cd outflux from the lumen obeys first order kinetics. These experiments were carried out at higher Cd concentrations than used here. Although our results provide no basis for such an assumption, it is possible that a second component of Cd translocation exists which does not become saturated in the concentration range studied. Evidence for two mechanisms of Cd uptake by duodenal tissue *in vitro* was also provided by Hamilton and Smith (1978). Koo *et al.*, (1978), in their work on transmural Cd movement out of the chicken intestine, studied Cd concentrations as high as 1 mM. Their conclusion that such Cd uptake could not be saturated is at variance with their observation of partial saturation over the concentration range of 0.01 to 0.10 mM. In addition, fractional Cd accumulation in duodenal tissue was clearly depressed by higher Cd concentrations. In any case, the saturable component studied here appears to be associated with mucosal cell membranes. This conclusion is based on the reversibility of saturation by simple dilution, a procedure which does not lead to significant washout of Cd from the tissue (Fig. 3). Work is in progress to determine whether the Cd accumulated in the tissue is concentrated in mucosal cells.

The great variability encountered in the kinetic analysis of Cd movement has so far precluded attempts to distinguish between competitive and non-competitive inhibition of Cd uptake by Ca. Calcium uptake by duodenal mucosa *in vitro* was reported to be non-competitively inhibited by Cd (Hamilton and Smith, 1978). Inversely, Ca exerted no effect on Cd uptake in this study, but, as in the work of Kojima and Kiyozumi quoted above, relatively very high Cd levels were employed.

Inhibition of Cd transport by milk illustrates the expected critical importance of the composition of luminal fluid. An influence of diet on Cd uptake into the body has often been reported, but it must be emphasized that changes in net, long-term Cd retention may not be related to acute effects of dietary constituents on unidirectional Cd flux into the gut wall. Thus, present results on the inhibition of Cd uptake by milk do not contradict the increased net Cd retention seen in young rats on a high milk diet by Kello and Kostial (1977).

Ability to transport Cd varies markedly along the jejunum (Fig. 1). Similar activity gradients for other solutes are well known. Further work will be necessary to explore the contributions of duodenum and ileum to total Cd transport.

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